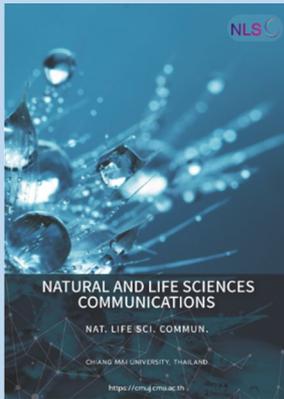


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Optimization of Gelatin Gummy Properties Influenced by Black Garlic Vinegar and Honey Using D-Optimal Mixture Design

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ABSTRACT

Black garlic is a valuable and versatile food product derived from the fermentation of fresh garlic. Black garlic possesses stronger antibacterial properties and contains antioxidants at levels twice as high as those found in regular garlic. The fermentation of black garlic into vinegar enhances its beneficial properties, including antimicrobial and antidiabetic effects. Currently, the market prospects for functional gummy candies are highly promising. This study aimed to optimize the formulation of functional gelatin gummy incorporating black garlic vinegar, gelatin and honey using a D-optimal mixture design. Product analysis included physicochemical, textural, and sensory properties of the gummies, evaluation of consumer acceptance and purchase intention. The optimal formulation for black garlic vinegar with honey gummies involved 10% black garlic vinegar, 17.06% gelatin, and 27.94% honey. Three experiments were conducted under practical conditions, yielding an average pH of 3.51 ± 0.02 , hardness of 18.21×10^3 g force, and gumminess of 16.44×10^3 g force. The sensory descriptive analysis provided the following ratings: appearance (7.30 ± 0.84), black garlic vinegar aroma (6.10 ± 1.39), honey aroma (6.54 ± 1.27), honey taste (7.24 ± 1.13), overall taste (7.18 ± 1.04), aftertaste (7.24 ± 1.19), and overall liking (7.26 ± 0.94). The final product received hedonic scores ranging from 6 to 7 for all attributes on a nine-point scale ($n=70$). Notably, 98% of consumers found the product acceptable, and 78% expressed an intention to purchase it. This indicates the successful development of a gelatin gummy product that effectively incorporates black garlic vinegar while maintaining a pleasant taste, making it appealing to a wide consumer base.

Keywords: Black garlic vinegar, Gelatin gummy, Mixture design, Texture analysis, Sensory analysis

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INTRODUCTION

Black garlic is a rapidly growing health food and the most studied. Its origins are uncertain, but it has been consumed in Korea, Japan, and Thailand since ancient times (Afzaal et al., 2021). In 2014, black garlic's market value was around \$94 million (Ahmed and Wang, 2021). Black garlic is aged garlic (*Allium sativum*) created through the Maillard reaction. This process occurs under high temperatures (60–90 °C) and humidity (70–90%). The aging process converts fresh garlic's harsh odor into a chewy texture and sweet taste. It also enhances the nutrients and bioactivity of black garlic (Najman et al., 2022). Black garlic contains bioactive substances, like phenol and organosulfur. These components offer various health benefits. They have potential therapeutic effects for many diseases. Studies show black garlic's bioactivities include antioxidation, anti-inflammation, and anti-obesity. It also has hepatoprotective, hypolipidemic, and anti-cancer properties. Black garlic can reduce allergies and modulate the immune system. It offers cardiovascular and neurodegenerative protection (Song et al., 2020).

Vinegar, an acidic condiment, has been utilized for over 3000 years (Li et al., 2021). It is produced using both solid-state and liquid-state fermentation methods. Depending on the raw materials, vinegar can be classified as either grain vinegar or fruit vinegar. Globally recognized as a fermented food, vinegar production methods vary by region (Ezemba et al., 2021). In European countries, vinegar is predominantly produced through liquid-state fermentation, examples include balsamic vinegar, sherry vinegar, and apple cider vinegar, using fruits like grapes, apples, tomatoes, persimmons, and pineapples as raw materials (Zhang et al., 2022). Conversely, in Asian countries, vinegar types such as Kurozu vinegar, Shanxi aged vinegar, Zhenjiang aromatic vinegar, and Baoning vinegar are typically brewed using solid-state fermentation. The raw materials for these vinegars consist of sorghum, wheat bran, beans, rice, and rice hulls. (Xia et al., 2020).

Black garlic vinegar is produced through the alcoholic or acetic fermentation of black garlic, using either the traditional Orleans method or the rapid (submerged) method commonly employed in industry, with various black garlic varieties (Kara et al., 2021). Recognized as safe (GRAS), vinegar is a natural food product free from additives (Rachmawati and Triwibowo, 2024). It is renowned for its diverse biological activities, including antioxidant, antibacterial, and antifungal properties. Polyphenols and organic acids, particularly acetic acid, play a crucial role in the beneficial properties of vinegar (Ousaaïd et al., 2021). Moreover, vinegar has been reported to offer various health benefits, including weight loss, the ability to lower blood glucose levels, and a reduced risk of heart disease. Animal studies have further elucidated the effects of vinegar consumption, enhancing our understanding of its beneficial effects and potential side effects (Launholt et al., 2020). Nowadays, many people consume vinegar directly (Perumpuli and Dilrukshi, 2022). Therefore, to provide consumers with more consumption options, this research has explored the application of black garlic vinegar in other product forms.

Gelatin is a water-soluble, high molecular weight polypeptide derived from the partial hydrolysis of collagen, characterized by its triple helix structure (You et al., 2020). Most gelatin is sourced from the skin, bones, and connective tissues of animals like cows, chickens, pigs, and fish. It is widely used as a thickening, stabilizing, foaming, and gelling agent in various food and pharmaceutical products (Rather et al., 2022). Confectionery gels (CGs), including gummy candies, are made with high sugar content, such as sucrose and glucose syrup, and are combined with gelling agents, food acids, flavorings, and colorings. Gelatin's firm, elastic nature makes it a preferred gelling agent in many gummy products at concentrations above 5% (w/w) (Wang and Hartel, 2022). At temperatures above the gelling point, gelatin chains exist in a flexible random coil conformation. As the gelatin gels, these chains undergo conformational transitions, partially regenerating the collagen triple helix structure, forming a thermoreversible network (Mosleh et al., 2023). Several factors, including

gelatin properties, concentration, pH, and temperature, influence the gelling process and thus the texture, which is crucial for gummies. The interactions between gelatin and other gummy ingredients also impact the physical properties and gelling process. For instance, sugars and polyols stabilize the gelatin gel, increasing its rigidity. Additionally, ionic strength and pH affect properties like turbidity and transmittance of the gelatin gel. Many gummy products on the market today are composite gels, containing two or more gelling agents, which contribute to specific flow behaviors, textures, appearances, and sensory properties (Ge et al., 2021). The confectionery market in Thailand is expected to expand by 5.37% from 2024 to 2028, reaching a market volume of USD 7.79 billion by 2028 (Statista, 2024). The gummy vitamins market in Thailand is experiencing substantial growth as consumers increasingly choose convenient and enjoyable alternatives to traditional supplement forms. These chewable and flavored gummies play a crucial role in delivering essential nutrients in a more palatable format (6Wresearch, 2024). According to the research of Osiriphun et al. (2022), gummy products account for over 50% of the candy market in Thailand. A gummy jelly is composed of gelatin, sugar, and glucose syrup, where gelatin provides the gelling function by forming a three-dimensional network, or junction zone, within the gel structure. Gummy jellies are not only easy to consume and transport but also attractive to athletes for energy intake during prolonged exercise (Wang et al., 2022). Additionally, their snack-like properties and potential health benefits make them appealing to young adults (Kitpot et al., 2020).

The objective of this study was to develop functional gummy product by optimizing the formulation of functional gelatin gummy incorporating black garlic vinegar, gelatin, and honey using a mixture design. The effects of these components on the physicochemical, textural and sensory properties of gummies were investigated. Additionally, consumer acceptance and purchase intention were evaluated.

MATERIALS AND METHODS

Materials

Black garlic vinegar (B-garlic, Noppada Products Co.,Ltd., Thailand), Gelatin (Imperial, Imperial (Thailand) Co., Ltd., Thailand, Sugar (MitrPhol, Mitr Phol Group, Thailand).

Black garlic vinegar with honey gummy formulation

The gelatin was initially mixed with room temperature water and then heated until dissolved into a clear solution. Subsequently, sugar and corn syrup were added to the mixture at 60–70°C and total soluble solids (TSS) were adjusted to 70–75°Brix. Finally, citric acid, black garlic vinegar, and honey were added. The mixture was immediately poured into a silicone mold. The gummy mixture was allowed to cool and set at room temperature for 2-4 hours before conducting a physicochemical analysis and sensory evaluation. The formulation base for the gelatin gummy is shown in Table 1.

Table 1. The formulation base for the gelatin gummy.

| Ingredients | g | % |
|-------------|-------|-------|
| Sugar | 19.80 | 13.75 |
| Corn syrup | 31.50 | 21.88 |
| Sorbitol | 13.20 | 9.17 |
| Citric acid | 2.00 | 1.69 |
| Water | 23.50 | 16.32 |

Preparation of black garlic vinegar with honey gummy using D-Optimal Mixture Design

The study aimed to develop black garlic vinegar with honey gummy using a composite blend formulation that incorporated various wall materials. These materials included black garlic vinegar (X1: 10-30%), gelatin (X2: 10-20%), and honey (X3: 10-30%). To achieve the desired quality in the encapsulated extracts, the formulation of the composite blend was optimized using a D-optimal mixture design with the assistance of Design-Expert software V.6.0.10 (Stat-Ease, Inc., USA) (Table 2).

Table 2. D-optimal mixture design for Mixture design for black garlic vinegar with honey gummy.

| Treatment | Black garlic vinegar | | Gelatin | | Honey | |
|-----------|----------------------|-------|---------|-------|-------|-------|
| | g | % | g | % | g | % |
| 1 | 15 | 10.42 | 10 | 6.94 | 30 | 20.83 |
| 2 | 30 | 20.83 | 15 | 10.42 | 10 | 6.94 |
| 3 | 20 | 13.89 | 15 | 10.42 | 20 | 13.89 |
| 4 | 10 | 6.94 | 20 | 13.89 | 25 | 17.36 |
| 5 | 25 | 17.36 | 20 | 13.89 | 10 | 6.94 |
| 6 | 30 | 20.83 | 10 | 6.94 | 15 | 10.42 |
| 7 | 10 | 6.94 | 15 | 10.42 | 30 | 20.83 |
| 8 | 20 | 13.89 | 15 | 10.42 | 20 | 13.89 |

Physicochemical properties analysis

Color

Color properties of the black garlic vinegar with honey gummy were determined by colorimeter and differences between color values of the black garlic vinegar with honey gummy samples were clarified in terms of L* (lightness), a* (red, green) and b* (blue, yellow) values (MiniScan EZ 45/0 (LAV), HunterLab, USA) (Yusuf et al., 2019). The measurement was done in triplicate.

pH

To measure the pH of the black garlic vinegar with honey gummy, the sample was heated with water at a 1:1 (w/w) ratio until it melted into a solution, then the pH was measured using a digital pH meter (Dilrukshi and Senarath, 2021).

Moisture content (MC)

The moisture content of the black garlic vinegar with honey gummy was measured by the AOAC method (AOAC, 2000). Samples of gummy (1 piece) were weighed and dried in an oven at 105°C until a constant weight was obtained. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following equation (1)

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where; W_1 = Sample weight before drying.

W_2 = Sample weight after drying.

Water activity (a_w)

The water activity meter (AquaLab TE3, Decagon Device, Inc., USA) was used to measure the a_w of the black garlic vinegar with honey gummy (Anuar et al., 2021).

Texture profile analysis (TPA)

The texture profile analysis (TPA) of black garlic vinegar and honey gummy (including hardness, cohesiveness, springiness, gumminess, and chewiness) was conducted following the method outlined by Renaldi et al. (2022), with 10 replications. The texture of the gummy was analyzed using a TA-XT plus texture analyzer (Stable Micro System, Surrey, UK) equipped with an SMS5 cylinder probe (50 mm). The analysis was carried out at room temperature with the following device parameters: pre-test speed of 1 mm/s, test speed of 5 mm/s, post-test speed of 5 mm/s, a strain of 75%, trigger force of 5 g, and a delay of 3 seconds between two compressions. All experiments were conducted in triplicate, and the resulting data were subjected to statistical analysis.

Sensory evaluation consumer test

The sensory evaluation of the black garlic vinegar with honey gummy was conducted using a 9-point hedonic scale with a series of nine categories ranging from 1 = extremely dislike to 5 = neither like nor dislike to 9 = like extremely. All of the attributes of gummy including appearance, color, black garlic vinegar aroma, honey aroma, overall aroma, black garlic vinegar taste, honey taste, overall taste, texture, aftertaste and overall liking were examined by 30 consumers (Lee et al., 2021). The optimal treatment was evaluated through a 9-point hedonic scale encompassing all attributes, as well as consumer acceptance and purchase intention, assessed by a sample of 70 consumers aged between 18 and 60. The panelists were selected based on three primary criteria: (1) affiliation with the Faculty of Agro-Industry, Chiang Mai University, Thailand, (2) willingness to try new ingredients and products and (3) regular consumption of gummy products.

Statistical analysis

The optimal mixture ratio of materials for black garlic vinegar with honey gummy was determined using a D-optimal mixture design. This specific experimental design method allows for the evaluation of various mixture combinations to identify the optimal ratio of the wall materials. Multiple regression analysis was conducted on the experimental data, resulting in the formulation of the following equation (2) to represent the model:

$$Y = b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_{12} \cdot x_1 \cdot x_2 + b_{13} \cdot x_1 \cdot x_3 + b_{23} \cdot x_2 \cdot x_3 + b_{123} \cdot x_1 \cdot x_2 \cdot x_3 \quad (2)$$

In the statistical analysis, the response variable is represented by Y, while b denotes the regression coefficients and x represents the independent variables. To determine the optimal mixture ratio of materials for the black garlic vinegar with honey gummy, which maximizes consumer acceptance, an optimization utilizing multiple response variables was performed using Design Expert software V.6.0.2 (Stat-Ease Co., USA). The least significant difference at $P \leq 0.05$ was calculated using the Duncan Multiple Range Test by SPSS V.17.0 software (IBM, New York, USA).

RESULTS

Physicochemical properties of black garlic vinegar with honey gummy

In a D-optimal mixture design experiment, black garlic vinegar, gelatin and honey were varied across three factors and three levels to create the gummy product. The resulting physicochemical characteristics are presented in Table 3.

Table 3. Physicochemical properties of black garlic vinegar with honey gummy.

| Trt. | Color | | | pH | a _w | MC (%) |
|------|---------------------------|--------------------------|---------------------------|--------------------------|---------------------------|---------------------------|
| | L* | a* | b* | | | |
| 1 | 59.87 ± 1.60 ^a | 2.35 ± 0.24 ^c | 7.38 ± 0.67 ^d | 2.93 ± 0.03 ^e | 0.76 ± 0.01 ^e | 26.98 ± 0.10 ^e |
| 2 | 55.09 ± 0.50 ^c | 2.82 ± 0.09 ^b | 8.33 ± 0.07 ^{bc} | 3.33 ± 0.04 ^d | 0.78 ± 0.01 ^c | 29.85 ± 0.23 ^c |
| 3 | 61.00 ± 0.71 ^a | 1.91 ± 0.16 ^d | 8.92 ± 0.12 ^{ab} | 3.34 ± 0.03 ^d | 0.76 ± 0.01 ^d | 28.14 ± 0.22 ^d |
| 4 | 54.91 ± 0.89 ^c | 3.98 ± 0.06 ^a | 8.66 ± 0.53 ^{bc} | 3.53 ± 0.02 ^b | 0.80 ± 0.01 ^a | 31.14 ± 0.22 ^b |
| 5 | 54.90 ± 0.22 ^c | 2.26 ± 0.20 ^c | 9.50 ± 0.35 ^a | 3.67 ± 0.01 ^a | 0.79 ± 0.01 ^b | 29.31 ± 0.48 ^c |
| 6 | 55.16 ± 0.17 ^c | 3.05 ± 0.26 ^b | 4.40 ± 0.14 ^f | 2.97 ± 0.08 ^e | 0.73 ± 0.01 ^f | 29.62 ± 0.33 ^c |
| 7 | 57.82 ± 0.32 ^b | 3.73 ± 0.10 ^a | 8.23 ± 0.19 ^c | 3.42 ± 0.01 ^c | 0.74 ± 0.01 ^f | 30.70 ± 0.40 ^b |
| 8 | 58.47 ± 0.15 ^b | 3.82 ± 0.04 ^a | 6.52 ± 0.02 ^e | 3.35 ± 0.02 ^d | 0.78 ± 0.01 ^{bc} | 33.23 ± 0.27 ^a |

Note: The average ± standard deviation with different letters in the column is significantly different ($P \leq 0.05$).

Table 3 shows the physical and chemical properties of the black garlic vinegar with honey gummy, including color, pH, a_w and percentage of moisture content (MC). The color was quantified using L*, a*, and b* values, where L* represents the range from black (0) to white (100), a* ranges from green (–) to red (+), and b* ranges from blue (–) to yellow (+). All treatments exhibited L* values ranging from 54.90 to 61.00, with the highest value observed in treatment 3, followed by treatments 8 and 7. The a* values ranged between 1.91 and 3.82, while b* values varied from 4.40 to 9.50, with the highest value recorded in treatment 5, followed by treatments 3 and 2. The water activity of the samples ranged from 0.73 to 0.80, with treatment 5 showing the highest value, followed by treatments 4 and 7. The moisture content of the gummy samples was found to be between 26.98% and 33.23%, with treatment 8 having the highest moisture content, followed by treatments 4 and 7.

Texture profiles are crucial factors influencing consumer acceptability. Table 4 presents the texture profiles of gummy jelly formulated with varying ratios of black garlic vinegar, gelatin, and honey. The texture characteristics examined in this study included hardness, springiness, cohesiveness, gumminess, and chewiness. The results revealed statistically significant differences ($P < 0.05$) across all texture attributes. Hardness was highest in treatment 5 (21051.68 ± 38.75 g force), followed by treatments 3 and 4, with values of 17936.13 ± 187.56 and 17843.58 ± 125.23 g force, respectively. The highest springiness was observed in treatment 6 (5.68 ± 0.20 g force), followed by treatments 4 and 2, which measured 5.45 ± 0.13 and 4.02 ± 0.40 g force, respectively. Treatment 4 exhibited the highest cohesiveness (0.93 ± 0.04 g force), followed by treatments 6 and 8 at 0.91 ± 0.01 and 0.90 ± 0.01 g force, respectively. Gumminess was highest in treatment 5 (18,887.09 ± 61.13 g force), with treatments 4 and 3 at 16,094.23 ± 5.06 and 15,727.80 ± 243.93 g force, respectively. Lastly, chewiness was highest in treatment 4 (90,225.18 ± 2,343.11 g force), followed by treatments 5 and 8, which measured 84,112.36 ± 5,593.47 and 74,871.00 ± 2,001.09 g force, respectively.

Table 4. Texture analysis of the black garlic vinegar with honey gummy.

| Trt | Hardness | Springiness | Cohesiveness | Gumminess | Chewiness |
|-----|---------------------------------|--------------------------|---------------------------|---------------------------------|-----------------------------------|
| | (g Force) | | | | |
| 1 | 11,707.68 ± 101.43 ^f | 2.36 ± 0.60 ^c | 0.87 ± 0.02 ^c | 10,407.24 ± 163.47 ^h | 26,770.94 ± 2,049.03 ^e |
| 2 | 16,145.72 ± 34.29 ^d | 4.02 ± 0.40 ^b | 0.88 ± 0.01 ^{bc} | 14,383.78 ± 137.30 ^e | 60,393.37 ± 2,764.36 ^d |
| 3 | 17,936.13 ± 187.56 ^b | 1.03 ± 0.02 ^d | 0.90 ± 0.01 ^{bc} | 15,727.80 ± 243.93 ^c | 15,531.48 ± 832.48 ^f |
| 4 | 17,843.58 ± 125.23 ^b | 5.45 ± 0.13 ^a | 0.93 ± 0.04 ^a | 16,094.23 ± 5.06 ^b | 90,225.18 ± 2,343.11 ^a |
| 5 | 21,051.68 ± 38.75 ^a | 1.06 ± 0.05 ^d | 0.90 ± 0.01 ^{bc} | 18,887.09 ± 61.13 ^a | 84,112.36 ± 5,593.47 ^b |
| 6 | 11,361.37 ± 118.50 ^g | 5.68 ± 0.20 ^a | 0.91 ± 0.01 ^{ab} | 11,677.94 ± 180.33 ^g | 58,810.47 ± 2,497.50 ^d |
| 7 | 17,563.87 ± 118.57 ^c | 1.11 ± 0.01 ^d | 0.90 ± 0.01 ^{bc} | 14,886.41 ± 80.46 ^d | 16,152.73 ± 379.57 ^f |
| 8 | 15,612.98 ± 65.68 ^e | 5.45 ± 0.22 ^a | 0.91 ± 0.01 ^{ab} | 13,477.90 ± 13.21 ^f | 74,871.00 ± 2,001.09 ^c |

Note: The average ± standard deviation with different letters in the column is significantly different ($P \leq 0.05$).

Sensory analysis revealed that the appearance, color, black garlic vinegar aroma, honey aroma, black garlic vinegar taste, honey taste, overall taste, texture, aftertaste, and overall liking of the black garlic vinegar with honey gummy exhibited significant differences ($P < 0.05$) between treatments (Table 5). The results indicated that black garlic vinegar decreased the liking scores for both aroma and taste in treatments 4, 7, 3 and 8, respectively. Conversely, the inclusion of honey increased the liking scores in treatments 4, 3, 7 and 1, respectively. These changes in the liking scores for aroma and taste significantly influenced the overall liking score of the black garlic vinegar with honey gummy. Treatments 7, 4, 3 and 8 exhibited the highest overall liking scores, with values of 6.70 ± 1.07 , 6.63 ± 1.08 , 6.27 ± 1.29 and 6.07 ± 1.44 , respectively. This suggests that consumers showed a preference ranging from slightly to moderately favorable towards these samples.

Table 5. Hedonic score from consumers of the black garlic vinegar with honey gummy.

| Attributes | Treatments | | | | | | | |
|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Appearance | 5.23 ± 1.71 ^b | 6.67 ± 1.35 ^a | 7.20 ± 1.11 ^a | 6.90 ± 1.19 ^a | 6.70 ± 1.27 ^a | 5.43 ± 1.67 ^b | 6.57 ± 1.33 ^a | 7.27 ± 1.12 ^a |
| Color | 5.60 ± 1.38 ^d | 6.97 ± 1.08 ^{ab} | 7.27 ± 1.03 ^a | 6.73 ± 1.15 ^{ab} | 6.67 ± 1.32 ^{ab} | 5.80 ± 1.66 ^{cd} | 6.40 ± 1.31 ^{bc} | 6.93 ± 1.00 ^{ab} |
| Black garlic vinegar aroma | 5.43 ± 1.00 ^{bc} | 4.87 ± 1.33 ^c | 5.80 ± 1.19 ^{ab} | 6.27 ± 1.06 ^a | 5.23 ± 1.52 ^{bc} | 4.73 ± 1.63 ^c | 5.67 ± 1.27 ^{ab} | 5.33 ± 1.66 ^{bc} |
| Honey aroma | 5.63 ± 1.11 ^{ab} | 4.90 ± 1.11 ^c | 5.90 ± 1.25 ^a | 6.10 ± 0.98 ^a | 5.13 ± 0.96 ^{bc} | 4.70 ± 1.35 ^c | 5.67 ± 1.25 ^{ab} | 5.20 ± 1.72 ^{bcd} |
| Black garlic vinegar taste | 5.50 ± 1.54 ^{bcd} | 4.80 ± 1.60 ^d | 6.07 ± 1.34 ^{abc} | 6.47 ± 1.26 ^a | 5.30 ± 1.39 ^{cd} | 4.80 ± 1.74 ^d | 6.23 ± 1.12 ^{ab} | 5.50 ± 1.63 ^{bcd} |
| Honey taste | 6.43 ± 1.28 ^a | 4.90 ± 1.33 ^c | 5.87 ± 1.56 ^{ab} | 6.57 ± 1.05 ^a | 5.13 ± 1.43 ^{bc} | 4.93 ± 1.63 ^c | 6.57 ± 1.20 ^a | 5.67 ± 1.49 ^{bc} |
| Overall taste | 6.17 ± 1.44 ^{ab} | 5.17 ± 1.51 ^{cd} | 6.13 ± 1.23 ^{ab} | 6.60 ± 1.17 ^{ab} | 5.27 ± 1.12 ^{cd} | 4.87 ± 1.59 ^d | 6.73 ± 1.03 ^a | 5.87 ± 1.38 ^{bc} |
| Texture | 6.33 ± 1.22 ^a | 6.10 ± 1.64 ^a | 6.30 ± 1.73 ^a | 6.23 ± 1.41 ^a | 5.07 ± 1.77 ^b | 6.30 ± 1.35 ^a | 6.67 ± 1.35 ^a | 6.67 ± 1.49 ^a |
| Aftertaste | 5.70 ± 1.19 ^{bcd} | 5.47 ± 1.12 ^{cd} | 6.17 ± 1.29 ^{abc} | 6.40 ± 1.05 ^{ab} | 5.17 ± 1.46 ^{de} | 4.73 ± 1.48 ^e | 6.73 ± 1.00 ^a | 6.10 ± 1.35 ^{abc} |
| Overall liking | 5.67 ± 1.27 ^{bcd} | 5.37 ± 1.28 ^{cde} | 6.27 ± 1.29 ^{ab} | 6.63 ± 1.08 ^a | 5.07 ± 1.31 ^{de} | 4.80 ± 1.51 ^e | 6.70 ± 1.07 ^a | 6.07 ± 1.44 ^{abc} |

Note: The average ± standard deviation with different letters in the row is significantly different ($P \leq 0.05$).

Optimization condition of the black garlic vinegar with honey gummy

The experimental data were subjected to multiple regression analysis to derive predictive equations for physicochemical properties (pH), texture profile (hardness and gumminess), and sensory attributes including aroma (black garlic vinegar and honey), taste (honey and overall), aftertaste, and overall liking. The equations, expressed in terms of coded values, are presented in Table 6.

Table 6. The models for the effects and interactions of the amount of Black garlic vinegar, gelatin and honey, using physicochemical and sensory attributes in conjunction with variable combinations.

| Dependent variable (Y) | Predictive model | R ² |
|----------------------------|---------------------------------------------------------------------------------|----------------|
| pH | $+2.36*X_1+5.93*X_2+2.32*X_3$ | 0.94 |
| Hardness | $+86.44*X_1+869.19*X_2+69.31*X_3$ | 0.84 |
| Gumminess | $+166.68*X_1+731.55*X_2+56.80*X_3$ | 0.88 |
| Appearance | $-0.11*X_1-0.72*X_2-0.15*X_3+0.33*X_1X_2+6.15\times 10^{-3}*X_1X_3+0.04*X_2X_3$ | 0.99 |
| Black garlic vinegar aroma | $+0.06*X_1+0.15*X_2+0.10*X_3$ | 0.85 |
| Honey aroma | $+0.06*X_1+0.13*X_2+0.11*X_3$ | 0.78 |
| Honey taste | $+0.05*X_1+0.12*X_2+0.15*X_3$ | 0.99 |
| Overall taste | $+0.05*X_1+0.14*X_2+0.14*X_3$ | 0.95 |
| Aftertaste | $-0.03*X_1-0.56*X_2+0.01*X_3+0.02*X_1X_2+3.50\times 10^{-4}*X_1X_3+0.03*X_2X_3$ | 0.99 |
| Overall liking | $-0.02*X_1-0.48*X_2-0.01*X_3+0.02*X_1X_2+1.35\times 10^{-3}*X_1X_3+0.02*X_2X_3$ | 0.99 |

Note: X₁; Black garlic vinegar, X₂; Gelatin, X₃; Honey

Table 6 presents the multiple regression results and the significance of regression coefficients for the pH, hardness, gumminess, black garlic vinegar aroma, honey aroma, honey taste, overall taste, aftertaste, and overall liking. The significance of each regression coefficient is indicated by its p-value, with lower P-values denoting higher significance. The table reveals that both the linear and quadratic terms of all parameters (black garlic vinegar, X₁; gelatin, X₂ and honey, X₃) have a significant effect on all attributes (at least at $P \leq 0.05$). Additionally, the interactions between the amounts of black garlic vinegar (X₁), gelatin (X₂), and honey (X₃) significantly influence all measured attributes ($P \leq 0.05$). However, increases in these ingredients are associated with decreases in appearance, aftertaste, and overall liking. The statistical analysis of hardness and gumminess (Table 6) indicated that a linear model was the most suitable ($P < 0.05$). However, the acceptability score in texture attribute (Table 5) was not significantly different ($P > 0.05$). Therefore, the results did not necessarily imply a direct impact of hardness and gumminess on consumer acceptability of the gummy's texture.

The relationships between the independent and dependent variables were visually depicted through 2D contour plots generated by the model (Figure 1a-j). These contour plots exhibited different shapes, indicating the nature of interactions between the variables. An elliptical contour plot suggested significant interactions between the variables, whereas a circular contour plot indicated the absence of significant interactions. These contour plots provided valuable insights into the relationships and interactions between the variables in the study.

In Figure 1, the interaction between the encapsulating wall materials namely black garlic vinegar (X₁), gelatin (X₂), and honey (X₃) was analyzed for the physicochemical properties and sensory attributes. The results indicated that an increase in black garlic vinegar, gelatin, and honey impacted the response variables. Specifically, an increase in black garlic vinegar and honey significantly influenced the sensory attributes. The texture profile, specifically hardness and gumminess,

increased proportionally with the gelatin concentration (Figure 1b-c). Additionally, the concentrations of black garlic vinegar and honey were identified as key factors, along with their interaction, in influencing the sensory attributes, including appearance, vinegar aroma, honey taste, overall taste, aftertaste, and overall liking (Figure 1d-j).

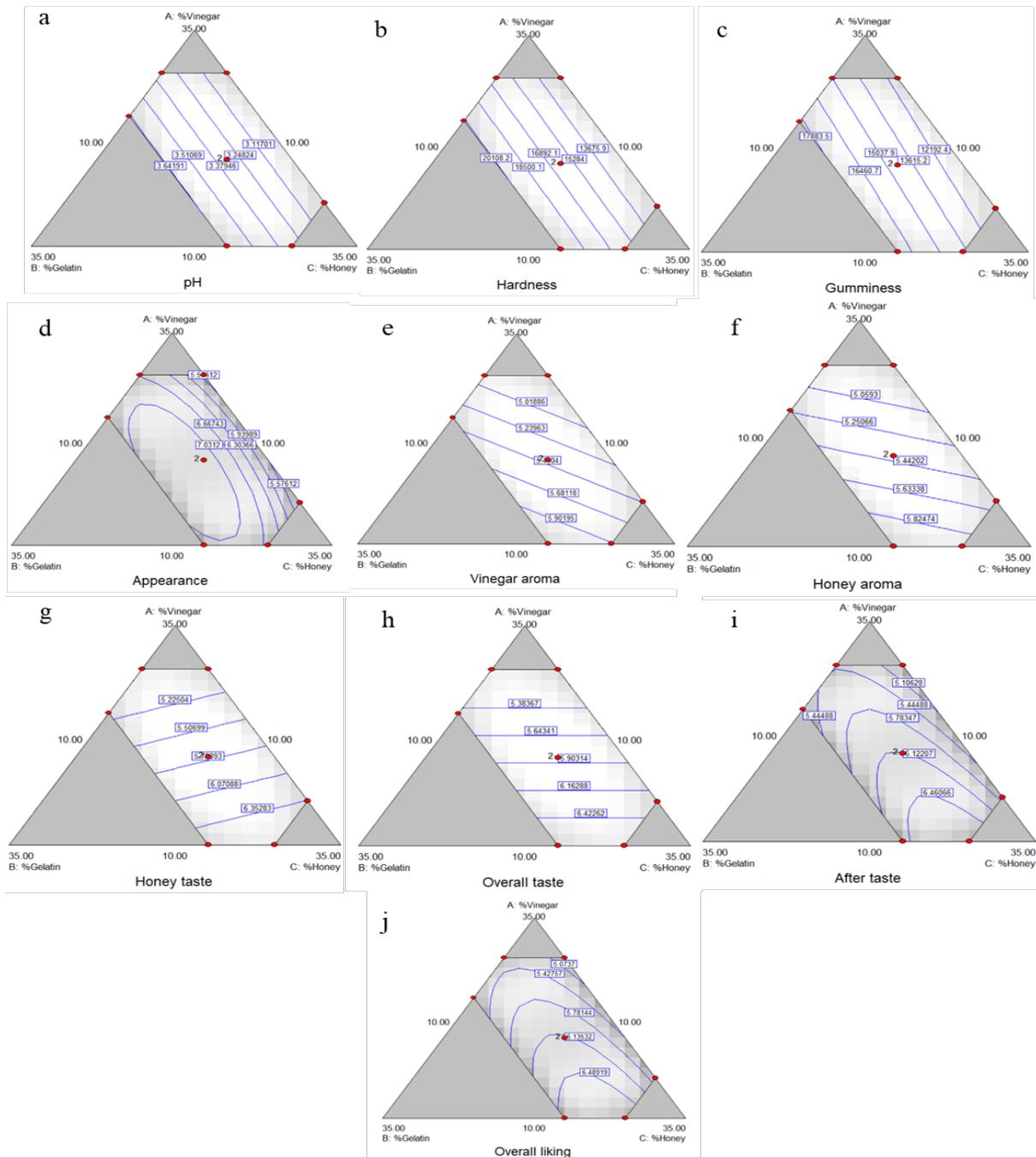


Figure 1. Contour plots showing the effect of the ratio of ingredients; A: black garlic vinegar, B: gelatin and C: honey on gummy's properties (a) pH, (b) hardness, (c) gumminess and sensorial attributes, (d) appearance, (e) black garlic vinegar aroma, (f) honey aroma, (g) honey taste, (h) overall taste, (i) after taste, and (j) overall linking.

The optimization of this study was to maximize the hedonic score for Appearance, Black garlic vinegar aroma, Honey aroma, Honey taste, Overall taste, Aftertaste and overall liking. Additionally, Figure 2 has low pH and texture (hardness and gumminess) for consumer acceptance. The optimal condition for gelatin gummy from black garlic vinegar with honey was determined using Design-Expert software, resulting in the following composition: 10 g of black garlic vinegar, 17.06 g of gelatin and 27.94 g of honey.

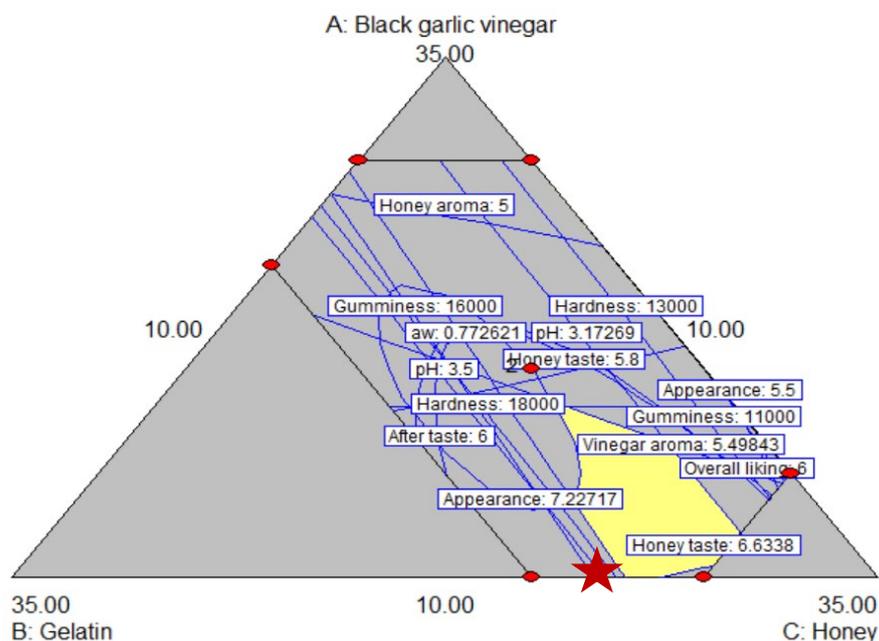


Figure 2. Overlay plot of optimum the condition of black garlic vinegar with honey gummy.

Verification of optimum condition of black garlic vinegar with honey gummy

The accuracy of predicting the optimal response values, which indicate the suitability of the optimized black garlic vinegar with honey gummy conditions, was evaluated by comparing the obtained experimental results with the post-analysis of the optimization procedure, as presented in Table 7.

Table 7. Comparison of the percentage of physicochemical and hedonic sensory analysis of black garlic vinegar with honey gummy of predicted value with the observed value.

| Attributes | Prediction value | Observe value | % Error |
|----------------------------|------------------|-------------------|---------|
| pH | 3.45 | 3.51 ± 0.02 | 1.74 |
| Hardness (g Force) | 17,630.60 | 18,205.97 ± 53.74 | 3.24 |
| Gumminess (g Force) | 15,235.10 | 16,443.03 ± 97.23 | 7.91 |
| Appearance | 6.93 | 7.30 ± 0.84 | 5.34 |
| Black garlic vinegar aroma | 6.00 | 6.10 ± 1.39 | 1.67 |
| Honey aroma | 5.96 | 6.54 ± 1.27 | 9.73 |
| Honey taste | 6.61 | 7.24 ± 1.13 | 9.53 |
| Overall taste | 6.68 | 7.18 ± 1.04 | 7.49 |
| After taste | 6.77 | 7.24 ± 1.19 | 6.94 |
| Overall liking | 6.84 | 7.26 ± 0.94 | 6.14 |

Note: The average ± standard deviation.

The optimal gelatin gummy condition for the black garlic vinegar with honey was determined using Design-Expert software, resulting in the following composition: 10 g of black garlic vinegar, 17.06 g of gelatin, and 27.94 g of honey. To validate practical applicability, three parallel experiments were conducted under these conditions. The average values for pH, hardness, gumminess, appearance, black garlic vinegar aroma, honey aroma, honey taste, overall taste, aftertaste, and overall liking were found to be 3.51 ± 0.02 , $18,205.97 \pm 53.74$ g force, $16,443.03 \pm 97.23$ g force, 7.30 ± 0.84 , 6.10 ± 1.39 , 6.54 ± 1.27 , 7.24 ± 1.13 , 7.18 ± 1.04 , 7.24 ± 1.19 , and 7.26 ± 0.94 , respectively, as shown in Table 7. It is noteworthy that the mean values closely align with the predicted values, indicating the reliability of the model.

Table 8. Hedonic score of black garlic vinegar with honey gummy (n = 70).

| Attributes | Linking score |
|----------------------------|-----------------|
| Appearance | 7.30 ± 0.84 |
| Color | 6.84 ± 1.27 |
| Black garlic vinegar aroma | 6.10 ± 1.39 |
| Honey aroma | 6.54 ± 1.27 |
| Black garlic vinegar taste | 6.84 ± 1.04 |
| Honey taste | 7.24 ± 1.13 |
| Overall taste | 7.18 ± 1.04 |
| Texture | 7.24 ± 1.19 |
| Aftertaste | 7.18 ± 1.04 |
| Overall liking | 7.26 ± 0.94 |

Note: The average \pm standard deviation.

Table 8 indicates that consumers' ratings on the 9-point hedonic scale for the black garlic vinegar with honey gummy fall within the range of 6-7. This suggests that consumers found the product moderately likable. Additionally, the consumer acceptance and purchase intention scores are notably high at 98% and 78%, respectively, as depicted in Figure 3.

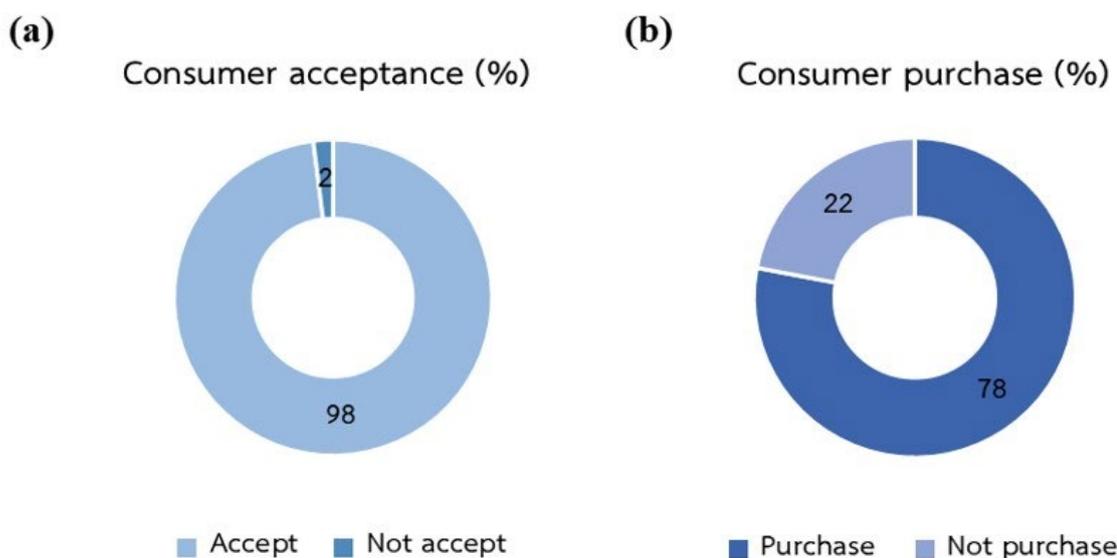


Figure 3. Percentage of consumer acceptance and purchase intention of black garlic vinegar with honey gummy.

DISCUSSION

The color of the gummy depends on the color of the black garlic vinegar. The dark tones of honey can also be due to the formation of Maillard reaction products as temperature increases (Becerril-Sánchez et al., 2021).

The water activity across all treatment variations was sufficiently low (0.73 to 0.80), providing microbial stability and ensure safe storage. Generally, microbial activity is inhibited at water activity levels below 0.6 for most fungi, below 0.7 for most yeast, and below 0.9 for most bacteria (Tapia et al., 2020).

Following previous studies (Teixeira-Lemos et al., 2021; Renaldi et al., 2022), typically, gummy jelly products have less than 20% moisture content. The observed higher moisture content in this study was attributed to the inclusion of sorbitol, which is known to retain moisture within the product (Klinmalai et al., 2021).

The optimal formulation ratio was determined using texture profile analysis, as these characteristics significantly impacted the rigidity of the gummy jelly formulation when combining the ingredients (Thilavech et al., 2023). The texture attributes that are particularly important for gelled confections include hardness, chewiness, and gumminess. Hardness is measured by the maximum force needed to deform the jellies during the first bite. Gumminess is determined by multiplying hardness by cohesiveness, while chewiness is calculated by multiplying hardness, cohesiveness, and springiness (Shahbazi et al., 2021). Hardness represents the strength of the gel structure when pressure is applied. An increase in gelatin and a decrease in pectin content significantly enhance the hardness of gummy jelly (Renaldi et al., 2022). Hardness is defined as the force needed to compress a food product by a specified amount. Springiness refers to the elastic recovery of the food when the compressive force is removed. Cohesiveness measures the strength of the internal bonds within the food sample. Chewiness quantifies the amount of energy required to chew a solid food product until it is ready for swallowing (Sumonsiri et al., 2021). Gumminess is derived from hardness and cohesiveness (A Abd EL Latif et al., 2022). The presence of co-solutes such as sugars, including polyols and other carbohydrates significantly influences the behavior of gelatin gels. Therefore, the sugar profile and concentration are crucial for the gelling properties of these systems. As explained by Wang and Hartel (2022), the mechanical properties, which we perceive as texture, of candies made with hydrocolloids, depend on the interaction between the gelling agents, water, and sugars, as well as the formation of junctions during the gelling process. The mechanisms that support gelation in gelatin gels containing sugars include (i) modification of the hydrogen-bonding water structure, (ii) reduction in the number of available water molecules due to the hydration of sugars, and (iii) exclusion of sugar molecules from the surface of polymeric hydrocolloid molecules, leading to their aggregation (Avallone et al., 2023).

Honey can blend and enhance foods' aroma, taste, flavor, and aftertaste (Habryka et al., 2021). Additionally, honey is commonly consumed as a natural medicine by many people. Incorporating honey into various foods could effectively increase its consumption (Leite et al., 2021). Moreover, white garlic's characteristic and pungent aroma, attributed to organosulfur compounds such as thiosulfate and sulfur volatiles, remains in black garlic (Kilic-Buyukkurt et al., 2023). This can affect the liking score in sensory tests.

Gelatin serves as the primary gelling agent that provides structure in gummy jellies (Jaroennon et al., 2023). The quantity of gelatin used in the formulation is approximately 12%, which aligns with the optimal amount identified in the research by Jiamjariyatam (2018). The high gelatin content is necessitated by the presence of black garlic vinegar, a fermentative product converted into acid (Singh, 2020). Acid reduces the stability of gelatin's structure (Wang et al., 2023). The maximum acid level at which gelatin can form a stable gel structure is at a pH of 2 (Samatra et al., 2022). The acids found in black garlic vinegar have been shown to effectively reduce the risks associated with diabetes, hypercholesterolemia, atherosclerosis,

hyperlipidemia, hypertension, inflammation, oxidative stress, cancer, and various neurodegenerative disorders (Afzaal et al., 2021). In contrast, commercial gummy candies generally contain high amounts of sucrose and glucose syrup, along with different gelling agents (Gok et al., 2020), hence they do not possess equivalent health benefit as compared to the newly developed product. The berries used in various commercial formulations, such as strawberries, raspberries, and blueberries, primarily provide anthocyanins and other phenolic compounds, which exhibit antioxidant activity (Teixeira-Lemos et al., 2021). The inclusion of honey in the product contributes to favorable sensory acceptance beside its contribution to micronutrients such as vitamins, mineral, antioxidants and monosaccharides. Therefore, the addition of honey can enhance the nutritional and sensory characteristics of the product (Leite et al., 2021).

The high acidity of the gummy results in reduced viscosity and gel strength. This is due to the hydrolysis of gelatin, which is exacerbated by decreased sugar inversion, leading to a weakened gelatin structure. In contrast, increasing the gelatin content enhances consumer satisfaction with the texture. Increasing gelatin concentration significantly affects the gel strength (Cheng et al., 2022), hence influencing the gummy's texture. The acceptability of foods, especially gummy products, is greatly influenced by their texture, as the mouthfeel of textural attributes significantly affects the eating experience (Vojvodić Cebin et al., 2024).

CONCLUSION

The optimal composition for the black garlic vinegar with honey gummy comprised 10 g of black garlic vinegar, 17.06 g of gelatin, and 27.94 g of honey. This final product received favorable hedonic scores, consistently ranging from 6 to 7 for all attributes on a nine-point hedonic scale. Additionally, 98% of consumers found the product acceptable, and 78% expressed an intention to purchase it. Despite increasing consumer awareness of the health-promoting properties of functional products, sensory acceptance remains the primary motivation for purchasing these items, even when they claim specific health benefits. However, further studies are necessary to conduct additional *in vitro* testing, specifically focusing on antioxidant and antidiabetic activities, in order to verify the potential health benefits of the functional food product.

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AUTHOR CONTRIBUTIONS

Threethip Chuensun performed the statistical analysis and drafted the manuscript. Pilairuk Intipunya procured research funding, designed and managed the project, conducted all experiments, assisted in teaching and guiding the gummy process, contributed to manuscript writing and edition. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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